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Research Article

Synthesis of an ^{18}F-labelled high affinity β_1-adrenoceptor PET radioligand based on ICI 89,406

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Summary

To date, some non-selective β-adrenoceptor (β-AR) positron emission tomography (PET) radioligands are in clinical use, but no PET radioligand for the selective imaging of cardiac β_1-ARs is clinically available. Therefore, the aim of this study was to develop a potential high-affinity PET radioligand for the β_1-subtype of ARs. Here, the synthesis and *in vitro* evaluation of (*S*)- and (*R*)-*N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-[4-(2-fluoro-ethoxy)-phenyl]-urea (**8a–b**), derivatives of the well-characterized β_1-AR selective antagonist, ICI 89,406, are described. The (*S*)-isomer **8a** shows both higher β_1-AR selectivity and β_1-AR affinity than the (*R*)-enantiomer **8b** (selectivity: 40 800 *vs* 1580; affinity: $K_{I1} = 0.049$ nM *vs* $K_{I1} = 0.297$ nM). Therefore, the ^{18}F-labelled analogue **8e** of compound **8a** was synthesized. While the direct nucleophilic ^{18}F-fluorination of the tosylate precursor **8d** produced **8e** in low radiochemical yields ($\leqslant$2.9% decay-corrected) and specific activities ($\leqslant$3.5 GBq/μmol at the end of synthesis (EOS), $n = 9$) the alternative two-step synthesis of **8e** from ethylene glycol *di-p*-tosylate **9**, [^{18}F]fluoride ion and phenol precursor **8f** gave satisfying results (16.4 $\pm$ 3.2% radiochemical yield (decay-corrected), 99.7 $\pm$ 0.5% radiochemical purity, 40 $\pm$ 8 GBq/μmol specific activity at the EOS within 174 $\pm$ 3 min from the end of bombardment (EOB) ($n = 5$)). Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: ICI 89,406 derivative; β_1-adrenoceptor selective ligand; PET radioligand

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Introduction

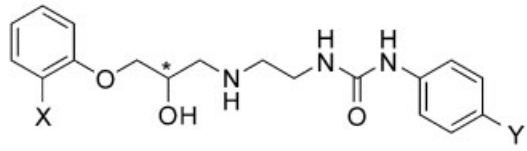
Postsynaptic β-adrenoceptors (β-ARs) are classified as rhodopsin/β_2-AR-like receptors that belong to one of three major subfamilies of the G-protein-coupled-receptors (GPCRs).[1] The β-AR family is subdivided into at least three discrete subtypes, the β_1-, β_2-AR[2] and the atypical β_3-AR.[3,4] Additionally, a putative subtype has been identified in cardiac tissue, classified as the β_4-AR.[5]

Within the left ventricle of the healthy human heart the β_1- to β_2-AR ratio is approximately 3:1.[6] In heart disease, both the β-AR density and the β_1- to β_2-AR ratio may change. Several conditions, including hypertension, heart failure, ischemia, hypertrophic and dilated cardiomyopathy (HCM, DCM) are accompanied by a reduced β-AR density in the heart.[7–12] In addition, the failing human heart is often characterized by a selective reduction in β_1-adrenoceptors (β_1-ARs) without change in β_2-AR density.[6]

For this reason a non-invasive method for the visualization and quantification of the β_1-AR density rather than total β-AR density in the human heart is of great interest in basic research and clinical application.[13] PET is an unique technique for this task, quantitatively and dynamically determining local radioactivity concentrations *in vivo* from which receptor concentrations can be calculated.[14] Non-selective radioligands for the imaging of β-ARs with positron emission tomography (PET), such as (*S*)-[^{11}C]CGP 12177, whose racemate was described by Delforge *et al.* in 1991,[15] were used in PET studies to quantify myocardial β-AR density in heart disease.[11,16,17] Elsinga *et al.* established (*S*)-[^{11}C]CGP 12388, a straightforward accessible radioligand, for non-selectively targeting β-ARs *in vivo*. In a recent study (*S*)-[^{11}C]CGP 12388 was administered as an aerosol for PET visualization of pulmonary β-ARs in healthy volunteers.[18] The radiosynthesis of an ^{18}F-labelled analogue of CGP 12388 has also been described.[19,20] In contrast, the clinical application of candidate β_1-AR selective radioligands, such as (+/−)-[^{11}C]HX-CH 44,[21] (*S*)-[^{11}C]bisoprolol,[22] or [^{11}C]CGP 20712A[23] and its (*S*)-enantiomer [^{11}C]CGP 26505,[24] is limited due to high non-specific binding, rapid metabolism or a tissue uptake that does not reflect binding to β-ARs.[21–24] In summary, no β_1-selective radioligand suitable for the non-invasive assessment of cardiac β_1-AR is clinically established for single photon emission computed tomography (SPECT) or PET.

An early example of a β_1-selective AR antagonist is the compound ICI 89,406 (Scheme 1). Membrane studies show that the (*S*)-enantiomer possess higher affinity than the antipode, but even the application of the racemate produces effective β_1-AR blockade during exercise in patients with angina pectoris.[30–32]

ICI 89,406 was chosen as the lead structure for the development of new β_1-selective AR radiotracers for application in nuclear medical imaging. After identification of several ICI 89,406 derivatives with high β_1-AR binding affinity[33] two β_1-AR radioligands labelled with ^{123}I and ^{125}I were synthesized



ICI 89,406: X = CN, Y = H, racemic;
I-ICI-H: X = ^{125}I or ^{123}I, Y = H, racemic;
I-ICI-COOH: X = ^{125}I or ^{123}I, Y = COOH, (*S*) or racemic;
ICI-OMe: X = CN, Y = O^{11}CH$_3$, (*S*) or (*R*)

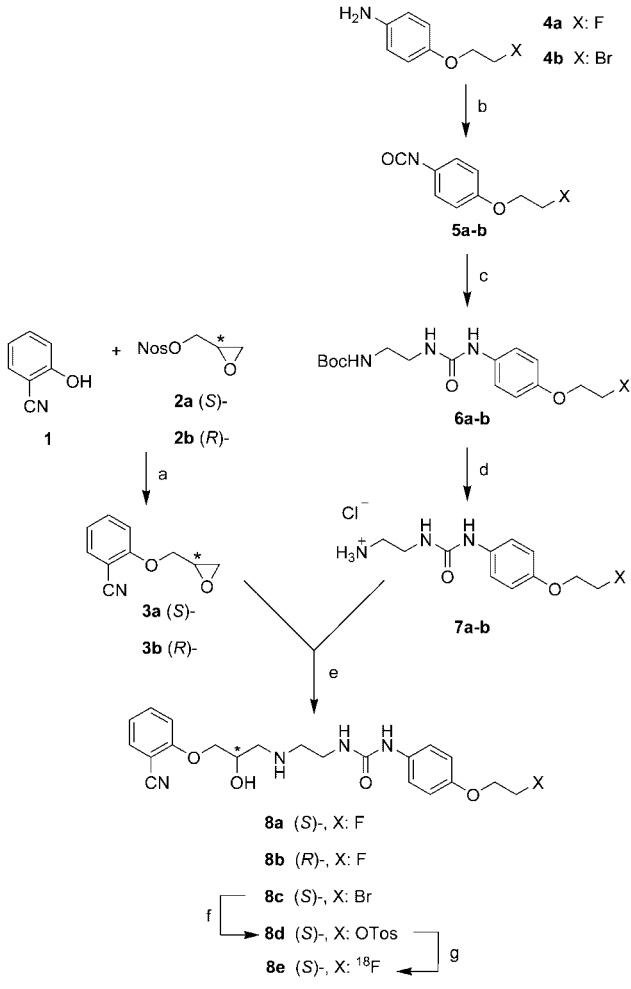
Scheme 1. Lead structure ICI 89,406 and its radiolabelled derivatives[25–29]

and evaluated *in vitro* and *in vivo* (I-ICI-H and I-ICI-COOH, Scheme 1). Drawbacks of these radioiodinated compounds regarding binding specificity and *in vivo* stability in the chosen animal models prevented further evaluation.[25,26] It seems that high non-specific binding and rapid deiodination *in vivo* preclude the use of such iodinated ligands for SPECT or PET. However, *in vitro* application of these radioligands seems possible, if ^{125}I-labelled analogues are considered, Radioligands labelled with positron-emitting ^{11}C ($t_{1/2} = 20.4$ min) or ^{18}F ($t_{1/2} = 109.7$ min) may be less susceptible to rapid metabolism. An ^{11}C-labelled ICI 89,406 derivative was recently introduced by our group as a potential high-affinity β_1-AR radioligand (ICI-OMe, Scheme 1).[27–29] At present this radioligand is being evaluated *in vivo*.

In a further step we aimed at the development of an ^{18}F-labelled ICI 89,406 derivative due to the superior physical decay characteristics (longer half-life, shorter average positron range) of ^{18}F compared to ^{11}C. The work presented here includes the synthesis of (*S*)-*N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-[4-(2-[^{18}F]fluoro-ethoxy)phenyl]-urea **8e** as a putative high affinity β_1-AR PET radioligand as well as the *in vitro* evaluation of the non-radioactive enantiomers **8a–b**.

Results and discussion

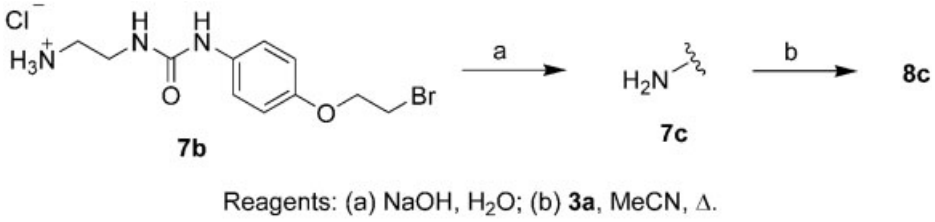
The ureas **8a–d**, that serve as precursor or reference compounds for the ^{18}F-labelled target **8e**, were synthesized in a seven- to nine-step sequence (Schemes 2 and 3). The nucleophilic substitution at the homochiral glycidyl-3-nitrobenzene sulphonates **2a** and **2b** with 2-cyano-phenol **1** under basic conditions provided the corresponding oxiranes **3a** and **3b** in good yields (85–92%). Comparison of the measured optical rotations with literature values showed that the stereochemistry was retained; the absolute $[\alpha]_D$ values were within errors.[34] The preparation of the second synthon, the hydrochlorides **7a–b**, started with the aniline derivatives **4a–b** that were prepared in two steps similar to those previously described (steps not shown in Scheme 2).[35–38] Compounds **4a–b** were converted into the corresponding



Reagents: (a) K_2CO_3, 2-butanone, Δ; (b) trichloromethyl chloroformate, ethyl acetate, Δ;
(c) *N*-mono-(*t*.-butyloxycarbonyl)ethylenediamine, diethylether, 0°C; (d) HCl, MeOH;
(e) NaOH, *n*-propanol, H_2O, Δ; (f) silver *p*-toluenesulfonate, MeCN, Δ;
(g) [^{18}F]K(Kryptofix 2.2.2)F, K_2CO_3, MeCN, Λ.

Scheme 2. Synthesis of the chiral β_1-AR ligands 8a–e

phenylisocyanates **5a–b** with trichloromethyl chloroformate (51–94%). The addition reaction of **5a–b** with *N*-mono-(*t*.-butyloxycarbonyl)ethylenediamine yielded the ureas **6a–b** (86–89%). After deprotection of **6a–b** that proceeded nearly quantitatively (94–96%) the target compounds **8a–c** were formed from the resulting hydrochlorides **7a–b** plus oxiranes **3a–b** via nucleophilic ring opening under basic reaction conditions. The chemical yields were low and ranged from 9 to 22% due to extensive purification procedures for the ureas **8a–c** (Scheme 2). An attempt to improve the yield of the bromide **8c** starting from the free amine **7c**, that was made from **7b** and NaOH, and oxirane **3b**



Scheme 3. Alternative synthesis of the chiral β_1-AR ligand 8c

Table 1. Inhibition constants and calculated β_1-AR selectivities of the ligands determined by a radioligand binding assay using mouse ventricular membrane preparations, plus calculated ligand log P and log D values

Compound	K_{I1} (nM)[a]	K_{I2} (nM)[a]	β_1-selectivity[b]	log P[c]	log D[c]
8a	0.049 ± 0.008	2000 ± 220	40 800 ± 7700	1.44	0.17
8b	0.297 ± 0.066	470 ± 180	1580 ± 550	1.44	0.17
8c	0.063 ± 0.036	890 ± 480	14 100 ± 780	1.98	0.71
8f[d]	0.328 ± 0.046	149 ± 27	453 ± 106	0.83	−0.55
ICI 89,406[d]	0.28 ± 0.12	41 ± 3	149 ± 86	1.57	0.13

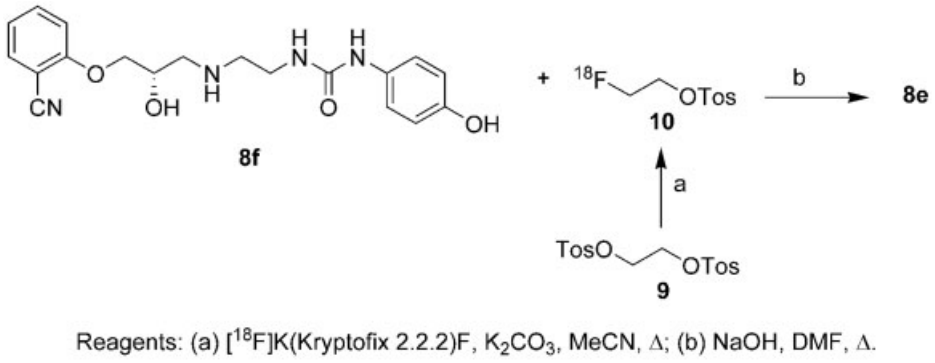
[a] Displacement of specifically bound non-selective β-AR ligand [^{125}I]ICYP binding at β_1- and β_2-ARs expressed in mouse ventricular membrane preparations as mean ± SEM, $n = 3$.
[b] The ratios of the low- over the high-affinity inhibition constants (K_{I2}/K_{I1}) indicate the β_1-selectivities of the non-radioactive β_1-AR ligands, noted as mean ± SEM, $n = 3$.
[c] log P values of the neutral form and log D values calculated by ACD/log D Suite (log D = log P at physiological pH 7.4 with consideration of charged species).
[d] from Reference[27].

did not succeed and provided, besides a low yield of urea **8c** (11%), mainly polymeric side products (Scheme 3). Finally, the bromide **8c** was converted with silver *p*-toluenesulphonate into the tosylate precursor **8d** in 46% yield (Scheme 2).

To assess the structure–activity relationships (SAR) between the ligands and β_1-ARs, competition studies using [^{125}I]iodocyanopindolol ([^{125}I]ICYP) and mouse ventricular membrane preparations were performed. The high- and low-affinity IC_{50} values for the β_1- and β_2-ARs of the non-radioactive 3-aryloxy-2-propanolamine derivatives **8a–c** were calculated by non-linear regression analysis of membrane bound radioactivity. From the IC_{50} values the high- and low-affinity inhibition constants (K_{I1} for the β_1-ARs and K_{I2} for the β_2-ARs) were obtained by the method of Cheng–Prusoff[39] using the previously determined K_D value of [^{125}I]ICYP (32.3 ± 1.9 pM)[33] (Table 1). The β_1-selectivities of the unlabelled compounds **8a–c** are defined by the ratios of the low- to high-affinity inhibition constants (K_{I2}/K_{I1}). To indicate the change in lipophilicities caused by the chemical modifications of the lead compound ICI 89,406 the calculated log P and log D values (ACD/log D Suite) of compounds **8a–c** are additionally listed in Table 1. The log P value of the non-radioactive target compounds **8a** and **8b** (log $P = 1.44$) is similar to the value

for (*S*)-CGP 12177 ($\log P = 1.81$)[40] whose ^{11}C-labelled counterpart is an effective radioligand for imaging cell surface β-ARs in the human myocardium with PET.[16,17] The compounds **8a–c** are characterized both by high β_1-AR binding affinities and β_1-AR selectivities. They possess β_1-AR affinities in the subnanomolar range ($K_{I1} = 0.049-0.297$ nM) and favour β_1-AR binding over β_2-AR binding resulting in high β_1-selectivities (1580–40 800). The enantiomers **8a** and **8b** display a normal binding behaviour for β_1-ARs, since the (*S*)-enantiomer **8a** possesses a higher affinity for this receptor subtype than the (*R*)-enantiomer **8b** ($K_{I1} = 0.049$ nM *vs* $K_{I1} = 0.297$ nM). Up to 100-fold higher β-AR affinities of (*S*)-enantiomers compared to the corresponding (*R*)-enantiomers have been documented.[41,42] A second feature of the enantiomer pair **8a** and **8b** is the 26-fold higher β_1-AR selectivity of **8a** (40 800 *vs* 1580). The (*S*)-enantiomer of the bromo substituted ligand **8c** shows similar β_1-AR affinity but nearly three times less β_1-AR selectivity than the (*S*)-fluoro counterpart **8a** ($K_{I1} = 0.063$ nM *vs* $K_{I1} = 0.049$ nM, 14 100 *vs* 40 800). Obviously a more sterically demanding halogen (covalent radius Br = 114 pm, covalent radius F = 72 pm) at the 2-ethoxy position in **8c** reduces the β_2-AR binding affinity less dramatically than the small-sized fluorine in **8a** resulting in a higher β_1-AR selectivity for **8a**. In summary, each ligand **8a–c** possesses higher β_1-AR binding affinities as well as β_1-AR selectivities than the racemic lead compound ICI 89,406 ($K_{I1} = 0.28$ nM, selectivity: 149).

As a result of these binding affinity and selectivity measurements, the more potent (*S*)-enantiomer **8a** (in comparison to its (*R*)-enantiomer **8b**) was chosen for conversion into a corresponding ^{18}F-labelled radioligand **8e** expected to have high affinity and selectivity for the β_1-AR subtype, for the use with PET. Two synthetic routes for the target compound **8e** were explored. The first one started with the tosylate precursor **8d** and [^{18}F]K(Kryptofix 2.2.2)F. Via nucleophilic substitution, the ^{18}F-labelled **8e** was obtained in one step (Scheme 2). This approach is however characterized by low radiochemical yields and specific activities ($\leqslant$2.9% (decay-corrected), $\leqslant$3.5 GBq/μmol at the end of synthesis (EOS), $n = 9$) that could not be improved by varying the reaction parameters (e.g. precursor concentration, reaction time and temperature). The specific activities may have been greatly underestimated due to incomplete HPLC-resolution of the radioligand **8e** from the non-radioactive by-products of the labelling procedure. Due to the low radiochemical yield this approach was not investigated further. The second approach (Scheme 4), a two-step synthesis, started with ethylene glycol *di-p*-tosylate **9** and [^{18}F]K(Kryptofix 2.2.2)F to yield 1-[^{18}F]fluoro-2-(tosyloxy)ethane **10** (radiochemical yield of 42.8 $\pm$ 2.8% (noted as mean $\pm$ SD), decay-corrected, $n = 5$). This well-known labelling synthon was used to alkylate the phenol compound **8f** under basic reaction conditions. Compound **8f** was prepared as previously described.[27] The second step provided the desired **8e** with a radiochemical yield of



Scheme 4. Alternative synthesis of the chiral β_1-AR radioligand 8e

$38.3 \pm 5.5\%$ (decay-corrected, $n = 5$). The overall radiochemical yield of **8e** for both steps was $16.4 \pm 3.2\%$ (decay-corrected). The synthesis was realized with a radiochemical purity of $99.7 \pm 0.5\%$ in 174 ± 3 min from end of bombardment (EOB) and a calculated specific radioactivity of 40 ± 8 GBq/μmol (30–50 GBq/μmol) at the EOS ($n = 5$). In summary, the two-step approach of **8e** provided more satisfying results regarding the radiochemical yield and specific radioactivity compared to the one-step procedure and is now being used to evaluate this ^{18}F-labelled compound in animals with PET.

Experimental

General methods

All chemicals, reagents, and solvents for the synthesis of the compounds were analytical grade and purchased from commercial sources. [^{125}I]ICYP was purchased from Perkin-Elmer.

The melting points (uncorrected) were determined on a Stuart Scientific SMP3 capillary melting point apparatus. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker ARX 300 and AMX 400 spectrometer, respectively. Mass spectrometry was performed via a Varian MAT 212 (EI = 70 eV) spectrometer and a Bruker MALDI-TOF-MS Reflex IV (matrix: DHB). Exact mass analyses were conducted on a Bruker MicroTof apparatus. $[\alpha]_D$ values were determined on a Perkin-Elmer 341 polarimeter. Elemental analysis was realized by a Vario EL III analyser. Radiosynthesis were partly carried out using an automated PET tracer synthesizer (TRACERLab Fx_{FDG} Synthesizer; GE Functional Imaging GmbH). The recorded data were processed by the TRACERLab Fx software (GE Functional Imaging GmbH). Separation of the radiosynthesized compounds was performed by gradient radio-HPLC using a Knauer K-500 and a Latek P 402 pump, a Knauer K-2000

UV-detector (wavelength 254 nm) and a Crismatec Na(Tl) Scintibloc 51 SP51 γ-detector, a Nucleosil 100-5 C18 column ($250 \times 4.6\,mm^2$) with a corresponding precolumn ($20 \times 4.0\,mm^2$) or a Nucleosil 100-10 C18 column ($250 \times 8\,mm^2$). Sample injection was carried out using a Rheodyne injector block (type 7125 incl. 200 µl or 1000 µl loop). The recorded data were processed by the NINA version 4.9 software (GE Functional Imaging GmbH). The radiochemical purities and the specific activities were acquired with a radio-HPLC system composed of a Syknm S1021 pump, a Knauer K-2501 UV-detector (wavelength 254 nm), a Crismatec Na(Tl) Scintibloc 51 SP51 γ-detector, a Nucleosil 100-3 C18 column ($200 \times 3\,mm^2$), a VICI injector block (type C1 incl. 20 µl loop) and the NINA version 4.8, Rev. 4 software (GE Functional Imaging GmbH).

Synthetic methods

Synthesis of oxiranes (**3a–b**)[27]

General procedure. 2-Cyano-phenol **1** (2 eq.), (*S*)-glycidyl-3-nitrobenzene sulphonate **2a** or (*R*)-glycidyl-3-nitrobenzene sulphonate **2b** (1 eq.) and anhydrous K_2CO_3 (6 eq.) were refluxed in dry 2-butanone (3.8 ml/mmol sulphonate) for 6–7 h and then stirred at RT for 16–72 h under an argon atmosphere. The mixture was filtered and the filter cake was washed with 2-butanone. The combined filtrates were evaporated to dryness. The residue was dissolved in water and CH_2Cl_2. The pH of the aqueous layer was adjusted to be within the range 11–13 and the aqueous layer was extracted. The layers were separated. The aqueous layer was twice extracted with CH_2Cl_2, the combined organic layers were dried (Na_2SO_4) and the solvent evaporated off. The residue was recrystallized from diisopropyl ether-$CHCl_3$ ($\sim$3:2 v/v) at $-30°C$ to provide **3a–b** as colourless solids.

(S)-2-(2-cyano-phenoxymethyl)-oxirane (**3a**). Yield: 92%. Mp.: 86°C; Lit.: 88–89°C[34]. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm]: 7.58–7.49 (m, 2H, H_{Aryl}), 7.10–7.01 (m, 2H, H_{Aryl}), 4.37 (dd, $^2J = 11.4$ Hz, $^3J = 3.0$ Hz, 1H, $1CH_2$), 4.12 (dd, $^2J = 11.4$ Hz, $^3J = 5.4$ Hz, 1H, $1CH_2$), 3.41–3.37 (m, 1H, CH), 2.93 ('t', $J = 4.5$ Hz, 1H, $1CH_2$), 2.84 (dd, $^2J = 5.0$ Hz, $^3J = 2.6$ Hz, 1H, $1CH_2$). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ [ppm]: 159.99, 134.35, 133.84, 121.42, 116.26, 112.80, 102.45, 69.47, 49.85, 44.54. $[\alpha]_D^{20} = +18.1°$ ($c = 1.0$, EtOH); Lit.: $[\alpha]_D^{25} = +17.7°$ ($c = 1.0$, EtOH).[34] Analytically calculated for $C_{10}H_9NO_2$: C 68.56, H 5.12, N 8.00. Found: C 68.80, H 4.96, N 8.38.

(R)-2-(2-cyano-phenoxymethyl)-oxirane (**3b**). Yield: 85%. Mp.: 85–86°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm]: 7.58–7.50 (m, 2H, H_{Aryl}), 7.09–7.01 (m, 2H, H_{Aryl}), 4.37 (dd, $^2J = 11.6$ Hz, $^3J = 3.2$ Hz, 1H, $1CH_2$), 4.12

(dd, $^2J = 11.6$ Hz, $^3J = 5.3$ Hz, 1H, $1CH_2$), 3.40–3.37 (m, 1H, CH), 2.93 (dd, $^2J = 4.8$ Hz, $^3J = 4.2$ Hz, 1H, $1CH_2$), 2.84 (dd, $^2J = 5.0$ Hz, $^3J = 2.4$ Hz, 1H, $1CH_2$). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ [ppm]: 160.01, 134.35, 133.87, 121.44, 116.26, 112.79, 102.50, 69.49, 49.85, 44.55. $[\alpha]_D^{20} = -15.8^\circ$ ($c = 1.0$, EtOH); Lit.: $[\alpha]_D^{25} = -16.0^\circ$ ($c = 1.0$, EtOH).[34] Analytically calculated for $C_{10}H_9NO_2$: C 68.56, H 5.12, N 8.00. Found: C 68.93, H 5.07, N 8.16.

Synthesis of the isocyanate derivatives (**5a–b**)

General procedure. A solution of 4-(2-fluoro-ethoxy)-aniline **4a**, prepared from 4-acetamido-phenol and toluene-4-sulphonic acid-(2-fluoro-ethyl ester) in two steps similar to those previously described,[35,36] or 4-(2-bromo-ethoxy)-aniline **4b**, prepared from 4-nitro-phenol and 1,2-dibromoethane in two steps as previously described,[37,38] in dry ethyl acetate (0.6–1.0 mmol/ml) was slowly added to a solution of 1 eq. trichloromethyl chloroformate in dry ethyl acetate (0.6 mmol/ml) at RT. The mixture was refluxed for 2 h and evaporated to dryness. The residue was distilled in a Kugelrohr to provide the phenyliso-cyanates **5a–b** as colourless oils.

4-(2-fluoro-ethoxy)-phenylisocyanate (**5a**). Yield: 94%. Bp. (1.2 mbar): $\leqslant 165^\circ$C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ [ppm]: 7.01 (dd, $^3J = 9.0$ Hz, $^4J = 0.9$ Hz, 2H, HAryl), 6.86 (dd, $^3J = 9.0$ Hz, $^4J = 0.9$ Hz, 2H, H_{Aryl}), 4.73 (dt, $^2J = 47.4$ Hz, $^3J = 4.2$ Hz, 2H, FCH_2), 4.18 (dt, $^3J = 27.6$ Hz, $^3J = 4.2$ Hz, 2H, CH_2O). ^{13}C-NMR (75.5 MHz, $CDCl_3$): δ [ppm]: 156.30, 126.66, 125.71, 115.65, 81.82 (d, $^1J = 170.4$ Hz), 67.56 (d, $^2J = 19.9$ Hz). ^{19}F-NMR (282 MHz, DMSO-d_6): δ [ppm]: −223.91. Analytically calculated for $C_9H_8FNO_2$: C 59.67, H 4.45, N 7.73. Found: C 59.55, H 4.20, N 7.65.

4-(2-bromo-ethoxy)-phenylisocyanate (**5b**). Yield: 51%. Bp. (1.4 mbar): 160–170°C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm]: 7.01 (d, $^3J = 9.0$ Hz, 2H, H_{Aryl}), 6.84 (d, $^3J = 9.0$ Hz, 2H, H_{Aryl}), 4.26 (t, $^3J = 6.6$ Hz, 2H, CH_2O), 3.62 (t, $^3J = 6.6$ Hz, 2H, $BrCH_2$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ [ppm]: 155.91, 126.79, 125.73, 115.65, 68.29, 28.84. MS (EI): *m/z* (intensity %): 243 ($M^{\bullet+}$, 100), 241 ($M^{\bullet+}$, 94), 135 (39), 134 (47), 109 (74), 107 (78), 106 (18). Analytically calculated for $C_9H_8BrNO_2$: C 44.66, H 3.33, N 5.79. Found: C 44.63, H 3.18, N 5.85.

Synthesis of the [2-[3-(4-(2-halogeno-ethoxy)-phenyl)-ureido]-ethyl]-carbamic acid t.-butyl ester derivatives (**6a–b**)

General procedure. 4-(2-Fluoro-ethoxy)-phenylisocyanate **5a** or 4-(2-bromo-ethoxy)-phenylisocyanate **5b** in dry diethyl ether (0.3–0.7 mmol/ml) was added slowly to 1 eq. *N*-mono-(*t*.-butyloxycarbonyl)ethylenediamine in dry diethyl ether (1.3–1.4 mmol/ml) at 0°C. The mixture was stirred for further 30 min at

0°C and cooled to −30°C for 1–14 h. After filtration the filter cake was washed with diethyl ether and dried *in vacuo* to provide [2-[3-(4-(2-halogeno-ethoxy)-phenyl)-ureido]-ethyl]-carbamic acid *t*.-butyl ester **6a–b** as colourless solids.

[2-[3-(4-(2-fluoro-ethoxy)-phenyl)-ureido]-ethyl]-carbamic acid t.-butyl ester (**6a**). Yield: 86%. Mp.: 163–164°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.29 (s, broad, 1H, NH), 7.28 (dt, $^{3}J = 9.0$ Hz, $^{4}J = 2.7$ Hz, 2H, H_{Aryl}), 6.83 (dt, $^{3}J = 9.3$ Hz, $^{4}J = 2.8$ Hz, 2H, H_{Aryl}), 6.78 (s, broad, 1H, NH), 6.03 (t, $^{3}J = 5.6$ Hz, 1H, NH), 4.69 (dt, $^{2}J = 47.7$ Hz, $^{3}J = 4.0$ Hz, 2H, FCH_2), 4.14 (dt, $^{3}J = 30.0$ Hz, $^{3}J = 3.9$ Hz, 2H, CH_2O), 3.11 (q, $^{3}J = 5.8$ Hz, 2H, CH_2), 2.99 (q, $^{3}J = 5.9$ Hz, 2H, CH_2), 1.37 (s, 9H, $C(CH_3)_3$). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 155.84, 155.62, 152.90, 134.20, 119.56, 114.82, 82.34 (d, $^{1}J = 166.6$ Hz), 77.75, 67.43 (d, $^{2}J = 19.1$ Hz), 40.69, 39.22, 28.36. ^{19}F-NMR (282 MHz, DMSO-d_6): δ [ppm]: −217.45. MS (MALDI-TOF): *m*/*z*: 364 $(M+Na)^+$. Analytically calculated for $C_{16}H_{24}FN_3O_4$: C 56.29, H 7.09, N 12.31. Found: C 56.12, H 6.99, N 12.41.

[2-[3-(4-(2-bromo-ethoxy)-phenyl)-ureido]-ethyl]-carbamic acid t.-butyl ester (**6b**). Yield: 89%. Mp.: 126–127°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.31 (s, 1H, NH), 7.27 (d, $^{3}J = 8.7$ Hz, 2H, H_{Aryl}), 6.83 (d, $^{3}J = 9.0$ Hz, 2H, H_{Aryl}), 6.77 (s, broad, 1H, NH), 6.04 (t, $^{3}J = 5.3$ Hz, 1H, NH), 4.23 (t, $^{3}J = 5.3$ Hz, 2H, CH_2O), 3.74 (t, $^{3}J = 5.6$ Hz, 2H, $BrCH_2$), 3.10 (q, $^{3}J = 6.0$ Hz, 2H, CH_2), 2.99 (q, $^{3}J = 5.8$ Hz, 2H, CH_2), 1.37 (s, 9H, $C(CH_3)_3$). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 155.65, 155.61, 152.60, 134.35, 119.51, 115.06, 77.74, 68.20, 40.66, 39.21, 31.66, 28.34. MS (EI): *m*/*z* (intensity %): 403 ($M^{\bullet+}$, 2), 401 ($M^{\bullet+}$, 94), 243 (100), 241 (100), 135 (28), 134 (32), 109 (67), 108 (51), 107 (69). Analytically calculated for $C_{16}H_{24}BrN_3O_4$: C 47.77, H 6.01, N 10.45. Found: C 47.82, H 6.13, N 10.53.

Synthesis of the N-(2-amino-ethyl)-N′-[4-(2-halogeno-ethoxy)-phenyl]-urea hydrochloride derivatives (**7a–b**)

General procedure. [2-[3-(4-(2-Fluoro-ethoxy)-phenyl)-ureido]-ethyl]-carbamic acid *t*.-butyl ester **6a** or [2-[3-(4-(2-bromo-ethoxy)-phenyl)-ureido]-ethyl]-carbamic acid *t*.-butyl ester **6b** was dissolved in a *c*. HCl-MeOH mixture (1:1 v/v, 0.6–0.7 mmol/ml). The solvents were evaporated *in vacuo* within 1 h. The residue was treated with 25 ml anhydrous acetone and the solvent was removed *in vacuo*. This procedure was repeated three times to provide the hydrochlorides **7a–b** as colourless solids that were dried *in vacuo*.

N-(2-amino-ethyl)-N′-[4-(2-fluoro-ethoxy)-phenyl]-urea hydrochloride (**7a**). Yield: 96%. Mp.: 204°C decomposition. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.91 (s, ^{1}H, NH), 8.09 (s, broad, 3H, NH_3^+), 7.31 (dt, $^{3}J = 9.3$ Hz, $^{4}J = 2.9$ Hz,

2H, H_{Aryl}), 6.83 (dt, $^3J = 9.0$ Hz, $^4J = 2.8$ Hz, 2H, H_{Aryl}), 6.64 (s, broad, 1H, NH), 4.68 (dt, $^2J = 47.7$ Hz, $^3J = 4.0$ Hz, 2H, FCH_2), 4.13 (dt, $^3J = 30.3$ Hz, $^3J = 3.9$ Hz, 2H, CH_2O), 3.31 ('d', $J = 4.5$ Hz, 2H, CH_2), 2.86 (q, $^3J = 5.8$ Hz, 2H, CH_2). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 156.18, 152.99, 134.07, 119.63, 114.82, 82.40 (d, $^1J = 166.7$ Hz), 67.42 (d, $^2J = 19.3$ Hz), 39.53, 37.34. ^{19}F-NMR (282 MHz, DMSO-d_6): δ [ppm]: −217.41. MS (MALDI-TOF): *m/z*: 264 $(M\text{-}HCl + Na)^+$, 242 $(M\text{-}HCl + H)^+$. Analytically calculated for $C_{11}H_{17}ClFN_3O_2$: C 47.57, H 6.17, N 15.13. Found: C 47.22, H 6.01, N 15.09.

N-(2-amino-ethyl)-N′-[4-(2-bromo-ethoxy)-phenyl]-urea hydrochloride (**7b**). Yield: 94%. Mp.: 176°C decomposition. ^{1}H-NMR (400 MHz, DMSO-d_6): δ [ppm]: 8.85 (s, 1H, NH), 7.99 (s, broad, 3H, NH_3^+), 7.31 (d, $^3J = 8.8$ Hz, 2H, H_{Aryl}), 6.82 (d, $^3J = 7.6$ Hz, 2H, H_{Aryl}), 6.55 (s, broad, 1H, NH), 4.22–3.73 (m, 4H, CH_2O and $BrCH_2$), 3.31–2.85 (m, 4H, $2CH_2$). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ [ppm]: 156.12, 152.74, 134.18, 119.65, 115.04, 68.22, 43.37, 39.55, 37.33. MS (MALDI-TOF): *m/z*: 326 $(M\text{-}HCl + Na)^+$, 324 $(M\text{-}HCl + Na)^+$, 304 $(M\text{-}HCl + H)^+$, 302 $(M\text{-}HCl + H)^+$. Analytically calculated for $C_{11}H_{17}BrClN_3O_2$: C 39.02, H 5.06, N 12.41. Found: C 38.87, H 5.17, N 12.56.

N-(2-amino-ethyl)-N′-[4-(2-bromo-ethoxy)-phenyl]-urea (**7c**). 14.00 g (41.3 mmol) *N*-(2-Amino-ethyl)-*N′*-[4-(2-bromo-ethoxy)-phenyl]-urea hydrochloride **7b** were dissolved in 150 ml water. The pH value was adjusted with 1 N NaOH to 9.0 and 200 ml CH_2Cl_2 were added. The aqueous layer was extracted and the two-phase mixture was filtered. The crude product on the filter was washed with 50 ml water and 50 ml CH_2Cl_2 and dried *in vacuo* over P_2O_5 to yield **7c** as a colourless solid. Yield: 82%. Mp.: 74–75°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm]: 7.33–7.26 (m, 2H, H_{Aryl}), 6.86–6.80 (m, 2H, H_{Aryl}), 6.33 (t, $^3J = 5.4$ Hz, 1H, NH), 6.18 (s, broad, 1H, NH), 4.24–3.73 (m, 4H, CH_2O and $BrCH_2$), 3.31–2.84 (m, 4H, $2CH_2$), 2.72 (t, $^3J = 5.2$ Hz, 2H, NH_2). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ [ppm]: 156.05, 152.80, 134.06, 119.75, 115.05, 68.20, 47.88, 39.81, 37.70. MS (MALDI-TOF): *m/z*: 304 $(M + H)^+$, 302 $(M + H)^+$. Analytically calculated for $C_{11}H_{16}BrN_3O_2 \cdot 0.5\ H_2O$: C 42.46, H 5.51, N 13.50. Found: C 42.81, H 5.48, N 13.72.

Synthesis of the N-aryl-N′-[2-[3-aryloxy-2-hydroxy-propylamino]-ethyl]-urea derivatives (**8a**–**c**)

General procedure of Scheme 2 (**8a**–**c**). 1 eq. **3a** or **3b**, an equimolar amount *N*-(2-amino-ethyl)-*N′*-[4-(2-halogeno-ethoxy)-phenyl]-urea hydrochloride **7a** or **7b** and 1.05 eq. of 10 N NaOH were heated in *n*-propanol (1.9–2.8 ml/mmol oxirane) and water (0.14–2.4 ml/mmol oxirane) up to 90°C for 3.5 h.

Purification procedure A (**8a–b**). The mixture was stored for crystallization at +4°C, the product was filtered off, washed with water (100 ml) and diethyl ether (100 ml), and was purified by silica gel chromatography (ethyl acetate–MeOH 4:1). The product fraction was evaporated and recrystallized from MeOH.

Purification procedure B (**8c**). Water was added (4.2 ml/mmol urea), the mixture was extracted with ethyl acetate three times, the combined organic layers were dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by silica gel chromatography (ethyl acetate–MeOH 4:1).

The urea derivatives **8a–c** were obtained as colourless solids.

Procedure of Scheme 3 (**8c**). 9.50 g (31.4 mmol) *N*-(2-Amino-ethyl)-*N′*-[4-(2-bromo-ethoxy)-phenyl]-urea **7c** were dissolved in 500 ml dry MeCN. Then 5.50 g (31.4 mmol) (*S*)-2-(2-cyano-phenoxymethyl)-oxirane **3a** were added. The solution was refluxed for 10 h. In the meantime, the mixture was decanted from the insoluble polymeric products three times. After 15 h stirring at RT it was refluxed for 5 h. The solution was evaporated at RT and the crude product was purified by silica gel chromatography (ethyl acetate–MeOH 4:1).

(S)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-[4-(2-fluoro-ethoxy)-phenyl]-urea (**8a**). Yield: 9%. Mp.: 142–143°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.32 (s, 1H, NH), 7.69 (dd, $^3J = 7.7$ Hz, $^4J = 1.7$ Hz, 1H, H_{Aryl}), 7.61 (ddd, $^3J = 8.9$ Hz, $^3J = 7.2$ Hz, $^4J = 1.5$ Hz, 1H, H_{Aryl}), 7.30–7.23 (m, 3H, H_{Aryl}), 7.06 (dt, $^3J = 7.7$ Hz, $^4J = 0.8$ Hz, 1H, H_{Aryl}), 6.82 (d, $^3J = 9.0$ Hz, 2H, H_{Aryl}), 6.04 (t, $^3J = 5.6$ Hz, 1H, NH), 5.01 (s, broad, 1H, OH), 4.69 (dt, $^2J = 47.8$ Hz, $^3J = 4.0$ Hz, 2H, FCH_2), 4.20–3.90 (m, 5H, $2OCH_2$ and CH), 3.14 (q, $^3J = 5.8$ Hz, 2H, CH_2), 2.76–2.60 (m, 4H, $2CH_2$), 1.93 (s, broad, 1H, NH). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 160.51, 155.65, 152.83, 135.13, 134.32, 133.77, 121.12, 119.41, 116.55, 114.83, 113.36, 100.81, 82.37 (d, $^1J = 166.6$ Hz), 71.77, 68.08, 67.42 (d, $^2J = 19.3$ Hz), 52.12, 49.50, 31.45. ^{19}F-NMR (282 MHz, DMSO-d_6): δ [ppm]: −217.36. MS (MALDI-TOF): *m/z:* 439 $(M + Na)^+$, 417 $(M + H)^+$. Analytically calculated for $C_{21}H_{25}FN_4O_4$: C 60.57, H 6.05, N 13.45. Found: C 60.22, H 5.94, N 13.35.

(R)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-[4-(2-fluoro-ethoxy)-phenyl]-urea (**8b**). Yield: 10%. Mp.: 140–141°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.32 (s, 1H, NH), 7.69 (dd, $^3J = 7.5$ Hz, $^4J = 1.8$ Hz, 1H, H_{Aryl}), 7.61 (ddd, $^3J = 8.6$ Hz, $^3J = 7.4$ Hz, $^4J = 1.4$ Hz, 1H, H_{Aryl}), 7.30–7.23 (m, 3H, H_{Aryl}), 7.06 (t, $^3J = 7.5$ Hz, 1H, H_{Aryl}), 6.83 (dt, $^3J = 8.7$ Hz, $^4J = 2.7$ Hz, 2H, H_{Aryl}), 6.04 (t, $^3J = 5.4$ Hz, 1H, NH), 5.00 (s, broad, 1H, OH), 4.69 (dt, $^2J = 47.8$ Hz, $^3J = 4.0$ Hz, 2H, FCH_2), 4.20–3.90 (m, 5H, $2OCH_2$

and CH), 3.14 (q, $^3J = 5.8$ Hz, 2H, CH_2), 2.75–2.60 (m, 4H, $2CH_2$), 1.91 (s, broad, 1H, NH). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 160.48, 155.60, 152.79, 135.09, 134.30, 133.74, 121.09, 119.39, 116.52, 114.81, 113.32, 100.80, 82.34 (d, $^1J = 165.5$ Hz), 71.73, 68.06, 67.42 (d, $^2J = 19.3$ Hz), 52.09, 49.48, 32.42. ^{19}F-NMR (282 MHz, DMSO-d_6): δ [ppm]: −217.59. MS (MALDI-TOF): *m/z*: 439 $(M+Na)^+$, 417 $(M+H)^+$. Analytically calculated for $C_{21}H_{25}FN_4O_4$: C 60.57, H 6.05, N 13.45. Found: C 60.42, H 6.31, N 13.33.

(S)-N-[4-(2-bromo-ethoxy)-phenyl]-N′-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea (**8c**). Yield: 22% (Scheme 2); 11% (Scheme 3). Mp.: 97–98°C. ^{1}H-NMR (300 MHz, DMSO-d_6): δ [ppm]: 8.33 (s, 1H, NH), 7.69 (dd, $^3J = 7.7$ Hz, $^4J = 1.7$ Hz, 1H, H_{Aryl}), 7.65–7.59 (m, 1H, H_{Aryl}), 7.26 ('t' $^3J = 9.0$ Hz, 3H, H_{Aryl}), 7.06 (t, $^3J = 7.5$ Hz, 1H, H_{Aryl}), 6.82 (d, $^3J = 9.0$ Hz, 2H, H_{Aryl}), 6.04 (t, $^3J = 5.4$ Hz, 1H, NH), 5.02 (s, broad, 1H, OH), 4.23 (t, $^3J = 5.4$ Hz, 2H, CH_2), 4.18–3.88 (m, 3H, CH_2CH), 3.74 (t, $^3J = 5.4$ Hz, 2H, CH_2), 3.15 (q, $^3J = 5.8$ Hz, 2H, CH_2), 2.77–2.62 (m, 4H, $2CH_2$). ^{13}C-NMR (75.5 MHz, DMSO-d_6): δ [ppm]: 160.47, 155.61, 152.53, 135.10, 134.46, 133.75, 121.11, 119.40, 116.53, 115.07, 113.33, 100.81, 71.22, 68.20, 67.96, 52.02, 49.34, 39.30, 31.68. MS (MALDI-TOF): *m/z*: 517 $(M+K)^+$, 515 $(M+K)^+$, 501 $(M+Na)^+$, 499 $(M+Na)^+$, 479 $(M+H)^+$, 477 $(M+H)^+$. Analytically calculated for $C_{21}H_{25}BrN_4O_4$: C 52.84, H 5.28, N 11.74. Found: C 53.13, H 5.25, N 11.58.

Toluene-4-sulphonic acid 2-[4-(3-{2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl}-ureido)-phenoxy]-ethyl ester (**8d**). Under an Ar-atmosphere 600 mg (1.26 mmol) (*S*)-*N*-[4-(2-bromo-ethoxy)-phenyl]-*N′*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-urea **8c** and 2.98 g (6.28 mmol) silver *p*-toluenesulphonate were refluxed in 80 ml dry MeCN for 70 h. The mixture was filtered and the filtrate was evaporated at RT. Then 30 ml of acetone was added, the mixture was stirred for 5 min, filtered and the filtrate was evaporated at RT. The crude product was purified by two silica gel chromatographies (ethyl acetate–MeOH 4:1) and **8d** was obtained as a pale yellow oil that solidified at 4°C. Yield: 46%. Mp.: 252°C decomposition. ^{1}H-NMR (400 MHz, $CDCl_3$): δ [ppm]: 7.76 (d, $^3J = 6.0$ Hz, 2H, H_{Aryl}), 7.68 (s, broad, 1H, NH), 7.46–7.41 (m, 2H, H_{Aryl}), 7.31 (d, $^3J = 6.0$ Hz, 2H, H_{Aryl}), 7.16 (d, $^3J = 6.6$ Hz, 2H, H_{Aryl}), 6.95 (t, $^3J = 5.7$ Hz, 1H, H_{Aryl}), 6.85 (d, $^3J = 6.6$ Hz, 1H, H_{Aryl}), 6.59 (d, $^3J = 6.9$ Hz, 2H, H_{Aryl}), 6.08 (s, broad, 1H, NH), 4.26–3.98 (m, 7H, $3OCH_2$ and CH), 3.39–3.34 (m, 2H, CH_2), 2.95–2.84 (m, 4H, $2CH_2$), 2.40 (s, 3H, CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ [ppm]: 160.35, 157.11, 153.96, 145.04, 134.72, 133.55, 129.94, 129.16, 128.01, 125.84, 121.74, 121.32, 116.76, 115.13, 112.63, 101.73, 68.37, 66.98, 65.93, 51.32, 49.59, 31.97, 29.73, 21.67. MS (ESI-EM): *m/z*: 569.2055 $(M+H)^+$

Calculated for $C_{28}H_{32}N_4O_7SH$ 569.2064; *m/z*: 591.1872 $(M+Na)^+$ Calculated for $C_{28}H_{32}N_4O_7SNa$ 591.1884.

Radiochemical experiments

Production of [^{18}F]fluoride ion and synthesis of [^{18}F]K(Kryptofix 2.2.2)F. No-carrier-added aqueous [^{18}F]fluoride ion was produced on a CTI-RDS-111 cyclotron by irradiation of a 1.2 ml water target using 10 MeV proton beams on 97.0% enriched [^{18}O]water by the $^{18}O(p,n)^{18}F$ nuclear reaction. A typical batch was 7.4 GBq of [^{18}F]fluoride ion at EOB for currents of 32 μA and irradiation time of 12 min. To recover the [^{18}O]water, the batch of aqueous [^{18}F]fluoride ion was passed through an anion exchange resin (Sep-Pak® Light Waters Accell™ Plus QMA cartridge, preconditioned with 5 ml 1 M K_2CO_3 and 10 ml water). [^{18}F]Fluoride ion was eluted from the resin with a mixture of 15 μl 1 M K_2CO_3, 200 μl water for injection, and 800 μl DNA-grade MeCN containing 15 mg Kryptofix® 2.2.2. Subsequently, the aqueous [^{18}F]K(Kryptofix 2.2.2)F solution was carefully evaporated to dryness *in vacuo*.

Radiosynthesis of (S)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-[4-(2-[^{18}F]fluoro-ethoxy)phenyl]-urea (**8e**) *via direct nucleophilic fluorination (one-step procedure).* Compound **8e** was prepared by treating 1.7 mg (2.98 μmol) tosylate precursor **8d** with the carefully dried [^{18}F]K(Kryptofix 2.2.2)F residue in 1 ml DNA-grade MeCN at 84°C for 5 min. After cooling to RT the crude reaction mixture was diluted with 10 ml water for injection and passed through a Waters Sep-Pak® Light C18 cartridge. The cartridge was washed with additional 10 ml water for injection, dried in a He-flow for 3 min, followed by elution of the raw **8e** with 1.5 ml MeOH. The solution was evaporated to a volume of 0.2 ml and fractionized using a gradient radio-HPLC procedure (conditions: $\lambda = 254$ nm; flow = 2 ml/min; eluents: A = MeCN-H_2O-TFA, 950/50/1, B = MeCN-H_2O-TFA, 50/950/1; column: Nucleosil 100-5 C18 ($250 \times 4.6\,mm^2$) with corresponding precolumn ($20 \times 4.0\,mm^2$); time programme: eluent B from 92 to 30% in 45 min, halt 5 min, from 30 to 92% in 8 min) resulting in **8e** with a radiochemical yield of 2.9% (decay-corrected) and a radiochemical purity >99% (retention time $R_t = 35.0$ min). The time of synthesis and purification was 91 min from the EOB. The determined specific radioactivity was 3.5 GBq/μmol at the EOS. The specific activity of **8e** was estimated by comparing the peak area of the UV channel of purified **8e** with a standard curve of known concentrations of reference compound **8a** realized with a RP-HPLC system (conditions: $\lambda = 254$ nm; flow = 0.3 ml/min; eluent: MeCN-H_2O-TFA, 700/300/1; column: Nucleosil 100-3 C18 ($200 \times 3\,mm^2$)). Chemical identity of **8e** was proved by

coinjection and coelution of **8e** and non-radioactive counterpart **8a** on the mentioned HPLC system.

Radiosynthesis of (S)-N-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-N′-[4-(2-[^{18}F]fluoro-ethoxy)phenyl]-urea (**8e**) *via 1-[^{18}F]fluoro-2-(tosyloxy)ethane* (**10**) *(two-step procedure)*. 4.0 mg (10.8 μmol) Ethylene glycol *di-p*-tosylate **9** and the carefully dried [^{18}F]K(Kryptofix 2.2.2)F residue were heated at 84°C in 1 ml DNA-grade MeCN for 4 min. The mixture was cooled to RT, diluted with 10 ml water for injection and passed through a Waters Sep-Pak® Light C18 cartridge. The cartridge was washed with additional 10 ml water for injection and eluted with 1.0 ml DNA-grade MeCN to yield raw **10**. The solution was purified using a gradient radio-HPLC procedure (conditions: $\lambda = 254$ nm; flow = 5 ml/min; eluents: A = MeCN-H_2O-TFA, 950/50/1, B = MeCN-H_2O-TFA, 50/950/1; column: Nucleosil 100-10 C18 (250×8 mm^2); time programme: eluent B from 92 to 30% in 20 min, halt 5 min, from 30 to 92% in 5 min). The product fraction of **10** (retention time $R_t = 19.4$ min) was evaporated to a volume of 2 ml, diluted with 10 ml water for injection and passed through a Waters Sep-Pak® Light C18 cartridge. After washing the cartridge with additional 5 ml water for injection it was dried in a He-flow for 10 min, followed by elution of **10** with 0.4 ml DMF that was tempered to 125°C before elution. Compound **10** was obtained with a radiochemical yield of 42.8 ± 2.8% (decay-corrected, $n = 5$). 4.0 mg (10.8 μmol) Compound **8f**, prepared as previously described,[27] in 0.1 ml DMF and 16.4 μl 1 N NaOH were added to the eluate of **10** (comment: the solution of **8f**, NaOH in DMF was prepared 3 h before reaction start and turned brown after 2 h). The reaction mixture was stirred at 125°C for 15 min, diluted with 0.5 ml water for injection and purified with a gradient radio-HPLC system (conditions: $\lambda = 254$ nm; flow = 5 ml/min; eluents: A = MeCN-H_2O-TFA, 950/50/1, B = MeCN-H_2O-TFA, 50/950/1; column: Nucleosil 100-10 C18 (250×8 mm^2); time programme: eluent B from 92 to 30% in 45 min, halt 5 min, from 30 to 92% in 5 min). After isolation of **8e** (retention time $R_t = 28.4$ min) the fraction was evaporated to a volume of 2 ml, diluted with 12 ml water for injection and passed through a Waters Sep-Pak® Light C18 cartridge. The cartridge was washed with 5 ml water for injection and eluted with 1 ml EtOH, that was tempered to 60°C before elution. For *in vivo* investigations the EtOH solution can be diluted with an appropriate amount of 0.9% NaCl or water for injection, respectively. In this second step the target compound **8e** was obtained with a radiochemical yield of 38.3 ± 5.5% (decay-corrected, $n = 5$). The overall radiochemical yield of **8e** for both steps was 16.4 ± 3.2% (decay-corrected). Target compound **8e** was prepared with a radiochemical purity of 99.7 ± 0.5% in 174 ± 3 min from EOB. The determined specific radioactivity was 40 ± 8 GBq/μmol at the EOS ($n = 5$) (see under one-step procedure).

Radioligand binding assay

Microsomes were prepared by homogenizing heart ventricles from DBA mice at 4°C for 90 s in buffer A (1 ml) containing 10 mM EDTA, 10 mM HEPES and 0.1 mM benzamidine (pH 7.4), using a Polytron PT 3000 (Kinematica, Lucerne, Switzerland). Homogenates were centrifuged at $45\,000 \times g_{max}$ for 15 min at 4°C. The pellets were resuspended again in buffer B (1 ml) containing 1 mM EDTA, 10 mM HEPES and 0.1 mM benzamidine (pH 7.4) and recentrifuged at $45\,000 \times g_{max}$ for 15 min at 4°C. The pellets were resuspended in buffer B (1 ml) and centrifuged at $10\,000 \times g_{max}$ for 10 min at 4°C. The supernatants were recentrifuged at $45\,000 \times g_{max}$ for 15 min at 4°C. The pellets, partially enriched membranes, were resuspended in buffer C (50 mM Tris · HCl, 5 mM $MgCl_2$ (pH 7.4)), and stored frozen at −80°C. For competition binding studies, the prepared membranes were resuspended in buffer D (10 mM Tris · HCl, 154 mM NaCl, 0.1 mM ascorbic acid, pH 7.4). 15 μg of membranes were incubated with a constant concentration of [^{125}I]ICYP (80 pM) and with varying concentrations (1 pM–100 μM) of compounds **8a–c** (Scheme 2). Reactions were conducted at 37°C for 60 min. Reactions were stopped by filtering onto Whatman GF/B filters and washed with water for injection. The membrane bound radioactivity was determined in a γ-scintillation counter. Competition binding curves were analysed by non-linear regression analysis as previously described[43–45] using the XMGRACE programme (Linux software). The data of the ligands **8a–c** fitted a two-site model significantly better than a one-site model ($F = 2.15$, $p < 0.05$). The resulting high- and low-affinity IC_{50} values of the non-radioactive 3-aryloxy-2-propanolamine derivatives **8a–c** were converted into the high- and low-affinity inhibition constants (K_{I1} for the β_1-ARs and K_{I2} for the β_2-ARs) by the method of Cheng–Prusoff[39] using the experimentally determined K_D value of [^{125}I]ICYP (32.3 ± 1.9 pM).[33] The ratios of the low- to high-affinity inhibition constants (K_{I2}/K_{I1}) yield the β_1-selectivities of the unlabelled derivatives **8a–c** (Table 1). These compounds show a significantly higher affinity to β_1- than to β_2-ARs. Additionally, the calculated log *P* and log *D* values (ACD/log *D* Suite) for compounds **8a–c** are listed in Table 1 to emphasise the changes of the lipophilicities caused by the chemical modifications of the lead compound, ICI 89,406.

Conclusion

The synthesis, radiosynthesis and *in vitro* pharmacology of (*S*)- and (*R*)-*N*-[2-[3-(2-cyano-phenoxy)-2-hydroxy-propylamino]-ethyl]-*N*′-[4-(2-fluoro-ethoxy)-phenyl]-urea (**8a** and **8b**, respectively), and the ^{18}F-labelled (*S*)-enantiomer (**8e**), a potential new high-affinity β_1-AR selective PET radioligand, are reported. The non-radioactive counterpart **8a** of the radiolabelled target

compound **8e** displays a high β-AR binding potency as well as a high β_1-AR selectivity in murine myocardial membranes and a moderate lipophilicity comparable with the known non-selective PET radioligand (*S*)-[^{11}C]CGP 12177. The radiosynthesis of **8e** was achieved in two alternative routes. The two-step synthesis of **8e** with 1-[^{18}F]fluoro-2-(tosyloxy)ethane **10** as intermediate shaped up as the superior route compared to the one-step procedure starting with the tosylate precursor **8d**. The two-step synthesis route yielded the target compound **8e** with reasonable radiochemical yields and specific activities. The formulation of the preparations is suitable for preclinical *in vivo* studies using small animal PET.

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